US-PAT-NO: 6150134
DOCUMENT-IDENTIFIER: US 6150134 A
TITLE: Purified hepatitis C virus envelope proteins for diagnostic and therapeutic use
DATE-ISSUED: November 21, 2000
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Maertens; Geert	Bruges	N/A	N/A	BEX
Bosman; Fons	Opwijk	N/A	N/A	BEX
De Martynoff; Guy	Waterloo	N/A	N/A	BEX
Buyse; Marie-Ange	Merelbeke	N/A	N/A	BEX

US-CL-CURRENT: 435/69.3,424/228.1 ,435/235.1 ,435/69.1 ,435/803 ,530/350

CLAIMS:

We claim:
1. A recombinant HCV single or specifically oligomerized envelope viral protein selected from the group consisting of E1, E2 or E1/E2 viral proteins obtained from a method comprising the steps of
(a) optionally lysing host cells expressing said viral protein;
(b) optionally recovering said viral protein;
(c) cleaving disulphide bonds of said viral protein with a disulphide bond cleaving agent to form a cleaved protein;
(d) preventing disulphide bond reformation of said cleaved viral protein with at least one of an SH group blocking agent and low pH condition; and
(e) purifying the cleaved viral protein obtained in step (d) to produce a viral protein which is at least 80% purified.
2. A recombinant protein according to claim 1 wherein said method further comprises desalting said purified viral protein of step (e).
3. A recombinant protein according to any one of claims 1 or 2 wherein step (b) or step (e) further comprises a chromatographic recovery.
4. A recombinant protein according to claim 3 wherein said affinity chromatography comprises lectin-chromatography or immunoaffinity chromatography with at least one of an anti-E1 specific monoclonal antibody or an anti-E2 specific monoclonal antibody.
5. A recombinant protein according to any one of claims 1 or 2 wherein said SH group blocking agent is N-ethylmaleimide.
6. A recombinant protein according to any one of claims 1 or 2, wherein said

purified protein is at least 95% pure.
7. A recombinant protein according to any one of claims 1 or 2, wherein said purified protein is at least 90% pure.
8. A recombinant protein according to claim 1 wherein step (b) further comprises an affinity chromatography.
9. A recombinant protein according to claim 1 wherein step (a) further comprises addition of an SH group blocking agent.
10. A recombinant protein according to claim 1 wherein said cleaving comprises partial cleaving conditions including addition of a detergent.
11. A recombinant protein according to claim 10 wherein said detergent comprises -N-Dodecyl-N,N-dimethylglycine.
12. A recombinant protein according to claim 11 wherein said detergent is present at a concentration of 1 to 10%.
13. A recombinant protein according to claim 11 wherein said detergent is present at a concentration of 3.5%.
14. A recombinant protein according to claim 1 wherein said disulphide bond cleaving agent is dithiothreitol.
15. A recombinant protein according to claim 14 wherein said dithiothreitol is present at a concentration of 0.1 to 50 mM.
16. A recombinant protein according to claim 15 wherein said dithiothreitol is present at a concentration of 0.1 to 20 mM.
17. A recombinant protein according to claim 15 wherein said dithiothreitol is present at a concentration of 0.5 to 10 mM.
18. A recombinant protein according to claim 1 wherein said purified protein is at least 97% pure.
19. A recombinant protein according to claim 1 wherein said purified protein is at least 98% pure.
20. A recombinant protein according to claim 1 wherein said purified protein is at least 99% pure.
21. A recombinant HCV single or specifically oligomerized envelope protein selected from the group consisting of E1, E2 or E1/E2 viral proteins, which is at least 80% pure.
22. A recombinant HCV single or specifically oligomerized envelope protein according to claim 21 which is at least 90% pure.
23. A recombinant HCV single or specifically oligomerized envelope protein according to claim 21 which is at least 95% pure.
24. A recombinant HCV single or specifically oligomerized envelope protein according to claim 21, which is at least 97% pure.
25. A recombinant HCV single or specifically oligomerized envelope protein according to claim 21, which is at least 98% pure.
26. A recombinant HCV single or specifically oligomerized envelope protein according to claim 21, which is at least 99% pure.

US-PAT-NO: 6245503
DOCUMENT-IDENTIFIER: US 6245503 B1
TITLE: Purified hepatitis C virus envelope proteins for diagnostic and therapeutic use
DATE-ISSUED: June 12, 2001
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Maertens; Geert	Bruges	N/A	N/A	BEX
Bosman; Fons	Opwijk	N/A	N/A	BEX
De Martynoff; Guy	Waterloo	N/A	N/A	BEX
Buyse; Marie-Ange	Merelbeke	N/A	N/A	BEX

US-CL-CURRENT: 435/5,424/204.1 ,435/69.3 ,435/7.1 ,435/810 ,435/975 ,530/300 ,530/350

CLAIMS:

What is claimed is:
1. Kit for detecting antibodies to HCV comprising:
at least one of an E1 protein and an E2 protein, said E1 protein and E2 protein having been purified to at least 80% pure; and
a buffer or components necessary for producing a buffer enabling formation of an immune complex between said protein and at least one of an anti-E1 antibody or anti-E2 antibody present in a biological sample, and
optionally, means for detecting said immune complex, and
optionally, at least one of an automated scanning or interpretation device for inferring a decrease of said anti-E1 antibody or anti-E2 antibody titers.
2. The kit according to claim 1 wherein said at least one protein is an E1 protein.
3. The kit according to claim 1 wherein said at least one protein is an E2 protein.
4. The kit according to claim 1, wherein said at least one protein is a purified recombinant HCV single or a specific oligomeric recombinant envelope protein selected from the group consisting of an E1 protein which has been purified to at least 80% pure and an E2 protein which has been purified to at least 80% pure.
5. The kit according to claim 4 wherein said recombinant protein had been expressed in recombinant mammalian cells.
6. The kit according to claim 4 wherein said recombinant protein had been expressed in recombinant yeast cells.
7. A kit according to claim 1 wherein said biological sample has been obtained from a patient receiving interferon.

8. A method for detecting antibodies to HCV comprising:
combining a sample which may contain said antibodies with at least one of an E1 protein of HCV which had been purified to at least 80% pure and an E2 protein of HCV which had been purified to at least 80% pure, and a buffer, under conditions such that an immune complex between said at least one protein and said antibodies present in said sample is formed; and
detecting said immune complex.
9. The method according to claim 8 wherein said at least one protein is an E1 protein.
10. The method according to claim 8 wherein said at least one protein is an E2 protein.
11. The method according to claim 8 wherein said at least one protein is a recombinant HCV single or a specific oligomeric recombinant envelope protein selected from the group consisting of an E1 protein which had been purified to at least 80% pure and an E2 protein which had been purified to at least 80% pure.
12. The method according to claim 11 wherein said recombinant protein had been expressed in recombinant mammalian cells.
13. The method according to claim 11 wherein said recombinant protein had been expressed in recombinant yeast cells.
14. The method according to claim 8 wherein said sample had been obtained from a patient receiving interferon.
15. Kit for detecting antibodies to HCV comprising an E1 protein of HCV and an E2 protein of HCV wherein at least one of said E1 protein and E2 protein has been purified to at least 80% pure; and
a buffer or components necessary for producing a buffer enabling formation of an immune complex between said protein and at least one of an anti-E1 antibody or anti-E2 antibody present in a biological sample, and
optionally, means for detecting said immune complex, and
optionally, at least one of an automated scanning or interpretation device for inferring a decrease of said anti-E1 antibody or anti-E2 antibody titers.
16. The kit according to claim 15 wherein each of said E1 protein and said E2 protein have been purified to at least 80% pure.
17. The kit according to claim 15 wherein at least one of said E1 protein of HCV and E2 protein of HCV is a purified recombinant HCV single or a specific oligomeric recombinant envelope protein which had been purified to at least 80% pure.
18. The kit according to claim 17 wherein said recombinant protein had been expressed in recombinant mammalian cells or recombinant yeast cells.
19. A method for detecting antibodies to HCV comprising:
combining a sample which may contain said antibodies with an E1 protein of HCV and an E2 protein of HCV wherein at least one of said E1 protein and said E2 protein has been purified to at least 80% pure, and a buffer, under conditions such that an immune complex is formed between said antibodies present in said sample and at least one of said E1 protein and said E2 protein; and

detecting said immune complex.

20. The method of claim 19 wherein each of said E1 protein and said E2 protein have been purified to at least 80% pure.

21. The method of claim 19 wherein at least one of said E1 protein of HCV and E2 protein of HCV is a purified recombinant HCV single or a specific oligomeric recombinant envelope protein which had been purified to at least 80% pure.

22. The method of claim 22 wherein said recombinant protein had been expressed in recombinant mammalian cells or recombinant yeast cells.

Restriction to one of the following inventions is required under 35 U.S.C. § 121. Because this application is filed under the provisions of #5 U.S.C. §371 restriction practice follows the requirements of PCT Rule 13.1 as modified by 37 CFR §1.475.

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under the provisions of 37 CFR §1.475. In addition, this application contains claims directed to more than one species which species are deemed to lack Unity of Invention because they are not so linked so as to form a single inventive concept.

Group I, claims 1-10 and 28, sharing the inventive concept of purifying HCV envelope proteins.

Group II, claims 11-13 and 27, sharing the inventive concept of purified HCV envelope proteins.

Group III, claims 15-22, 25 and 26 sharing the inventive concept of expression vectors for HCV envelope proteins.

Group IV, claims 23 and 24, sharing the inventive concept of being drawn to envelope protein encoding nucleotide sequences.

Groups V(a)-V(o), claim 29, each of the polyeptides represented by SEQ ID NO 56(a), 57(b), 58(c), 59(d), 53(e), 66(f), 67(g), 68(h), 72(i), 73(j), 86(k), 87(l), 88(m), 83(n) and 82(o) respectively share the inventive concept of being peptides from an HCV envelope protein, however, each of (a)-(o) appears to have a separate and unique sequence whose properties would appear to confer upon it biological properties which would not permit one of skill in the art to hold one obvious in view of any of the others.

Groups VI(F)-VI(I), claim 30, sharing the inventive concept of being drawn to conformational epitopes, however, there is no common special technical feature since each of the conformational epitopes has a unique structure.

Group VII, claim 31, sharing the inventive concept of being drawn to monoclonal antibodies directed against HCV envelope proteins.

Group VIII, claim 34, having the inventive concept of detecting HCV antigen using an anti-envelope monoclonal antibody.

Group IX, claim 35, having the inventive concept of a kit containing anti-envelope monoclonal antibodies.

Group X, claim 37, having the inventive concept of a method of vaccination.

Group XI, claims 38 and 39, having the inventive concept of a method of

vaccination employing HCV envelope proteins.

Group XII, claim 42, having the inventive concept of in vitro diagnosis of HCV antibodies employing HCV envelope proteins.

Group XIII, claims 43 and 47, having the inventive concept of kit containing HCV envelope proteins.

Group XIV, claim 44, having the inventive concept of a method of in vitro monitoring of HCV disease using E1 protein of HCV.

Group XV, claim 45, having the inventive concept of a kit containing at least one E1 polypeptide.

Group XVI, claim 46, drawn to a method of subtyping HCV employing one or more serological subtypes of HCV envelope proteins.

Group XVII, claim 48, drawn to an HCV envelope polypeptide immobilized on a strip.

With respect to unity of invention PCT Rule 13.1 states:

> The international application shall relate to one invention only or to a group of inventions so linked as to form a single general inventive concept ("requirement of unity of invention").

Additionally, PCT Rule 13.2 states:

> Unity of invention exists only when there is a technical relationship among the claimed inventions involving one or more special technical features. The term "special technical features" is defined as meaning those technical features that define a contribution which each of the inventions considered as a whole, makes over the prior art.

With regard to the application of PCT Rule 13, 37 CFR §1.475 concerning unity of invention states:

> (a) An international and a national stage application shall relate to one invention only or to a group of inventions so linked as to form a single general inventive concept ("requirement of unity of invention"). Where a group of inventions is claimed in an application, the requirement of unity of invention shall be fulfilled only when there is a technical relationship among those inventions involving one or more of the same or corresponding special technical features. The expression "special technical features" shall mean those technical features that define a contribution which each of the claimed inventions, considered as a whole, makes over the prior art.

(b) An international or a national stage application containing claims to different categories of invention will be considered to have unity of invention if the claims are drawn only to one of the following combinations of categories:

(1) A product and a process specially adapted for the manufacture of said product; or

(2) A product and a process of use of said product; or

(3) A product, a process specially adapted for the manufacture of the said product, and a use of the said product; or

(4) A process and an apparatus or means specifically designed for carrying out the said process; or

(5) A product, a process specially adapted for the manufacture of the said product, and an apparatus or means specifically designed for carrying out the said process.

(c) If an application contains claims to more or less than one of the combinations of categories of invention set forth in paragraph (b) of this section, unity of invention might not be present.

(d) If multiple products, processes of manufacture or uses are claimed, the first invention of the category first mentioned in the claims of the application and the first recited invention of each of the other categories related thereto will be considered as the main invention in the claims, see PCT Article 17(3)(a) and 1.476(c).

(e) The determination whether a group of inventions is so linked as to form a single general inventive concept shall be made without regard to whether the inventions are claimed in separate claims or as alternatives within a single claim.

The inventive concepts of Groups II-IV, VII, VIII-XIII, XV-XVIII does not appear to constitute a "special technical feature" within the meaning of PCT Rule 13.1 as they are either explicitly taught in Choo et al. (1994), Lanford et al. (1993), Watanabe et al. (US 5,610,009) or Bukh et al. (US 5,514,539) or obvious over their combined teachings. Each of the cited references is specifically concerned with the envelope proteins of HCV and the genes which encode them including vaccines, subtyping of HCV strains, and methods of detecting HCV antigens and anti-HCV antibodies.

Because these inventions lack unity of invention for the reasons given above restriction for examination purposes as indicated is proper.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to MP Woodward whose telephone number is (703) 308-3890. The examiner can normally be reached on Monday-Thursday and

alternate Fridays from 8:00 AM to 5:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Marian Knode, can be reached on (703) 308-4311.

The fax phone number for this Art Unit is (703) 305-7939.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

May 13, 2002